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Cytogenetic Evidence for a Less Malignant Leukemic Cell Population in the Central Nervous System in a Critical Case of Acute Myeloblastic Leukemia

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Background. With the exception of a single study the cytogenetic aspects of leukemic cells in the central nervous system (CNS) have not been investigated.

Patients and Results. During the course of a work-in-progress on the chromosomal constitution both of the spinal fluid and of bone marrow (BM) in children with acute myeloblastic leukemia (AML), we have observed a unique case of AML and CNS leukemia (CNSL) at diagnosis. The patient showed the simultaneous presence at diagnosis of a 46 cytogenetic line in the spinal fluid and a 47 (+8) cell line in the BM, present in the great majority of the metaphases examined.

Discussion. This observation allows hypotheses on the relationship between BM and CNS disease in AML. Regardless of the pathogenetic mechanism, the cytogenetic findings of the present case clearly suggest that the leukemic population in the CNS compartment represents a less malignant cell process compared to the BM leukemic population. This easily fits in with the usually less malignant course of CNSL in AML.

Conclusion. The foregoing findings may have critical pathogenetic and therapeutic implications. Med. Pediatr. Oncol. 30:91–94, 1998. © 1998 Wiley-Liss, Inc.

Key words: acute myeloblastic leukemia; meningeal leukemia; cytogenetics; chromosome; clonal evolution

INTRODUCTION

Little is known about the mechanisms leading to the development of central nervous system leukemia (CNSL), which is presumed to arise in the blood. CNSL has been considered a sanctuary for leukemic blasts since the blood-brain barrier may protect these cells from the action of systemically given drugs [1]. Patients with central nervous system (CNS) relapse often have subsequent bone marrow (BM) relapse, but it is only a postulation that the CNSL cells may be ″reseeding″ the BM. Thus the relationship between CNS disease and BM disease in acute leukemia is far from clear. Nevertheless, one of the most important factors contributing to the good results obtained in the treatment of children with acute lymphoblastic leukemia (ALL) has been the institution of CNS ″preventive″ therapy, with cranial radiation and/or intrathecal chemotherapy [1].

With improved survival also in children with acute myeloblastic leukemia (AML), CNSL has been diagnosed with increasing frequency. The results of ″prevention″ of CNSL in AML are more controversial than in ALL [2]. Improved understanding of the biological events concerning CNSL in children is needed and may provide better strategies for CNS ″prophylaxis″ and treatment both for ALL and, even more, for AML.

Surprisingly, chromosome studies in acute leukemia have been restricted to BM and cultured blood cells and the cytogenetic aspects of leukemic cells in the CNS have not been investigated, with the exception of a single study in 1970 [3].

During our present work-in-progress on the chromosomal constitution both of the spinal fluid and of BM in children with AML, we have observed a unique case of AML and CNSL at diagnosis. This case throws critical light on the pathogenesis of CNSL and BM leukemic infiltration in children with AML.

CASE REPORT

A 17-month-old girl was admitted to our center because of fever, slowly increasing swelling of the right mandibula, and hepatosplenomegaly of a few weeks duration. A week before admission a right facial palsy was noted. The patient had no remarkable past history and growth and development were normal. Physical examination revealed hepatosplenomegaly (liver 3 cm and spleen 4 cm below the costal margin); mild cervical and inguinal lymphoadenopathy; a 1 × 2 cm retromolar firm, tender swelling of the right mandible; and right facial palsy.

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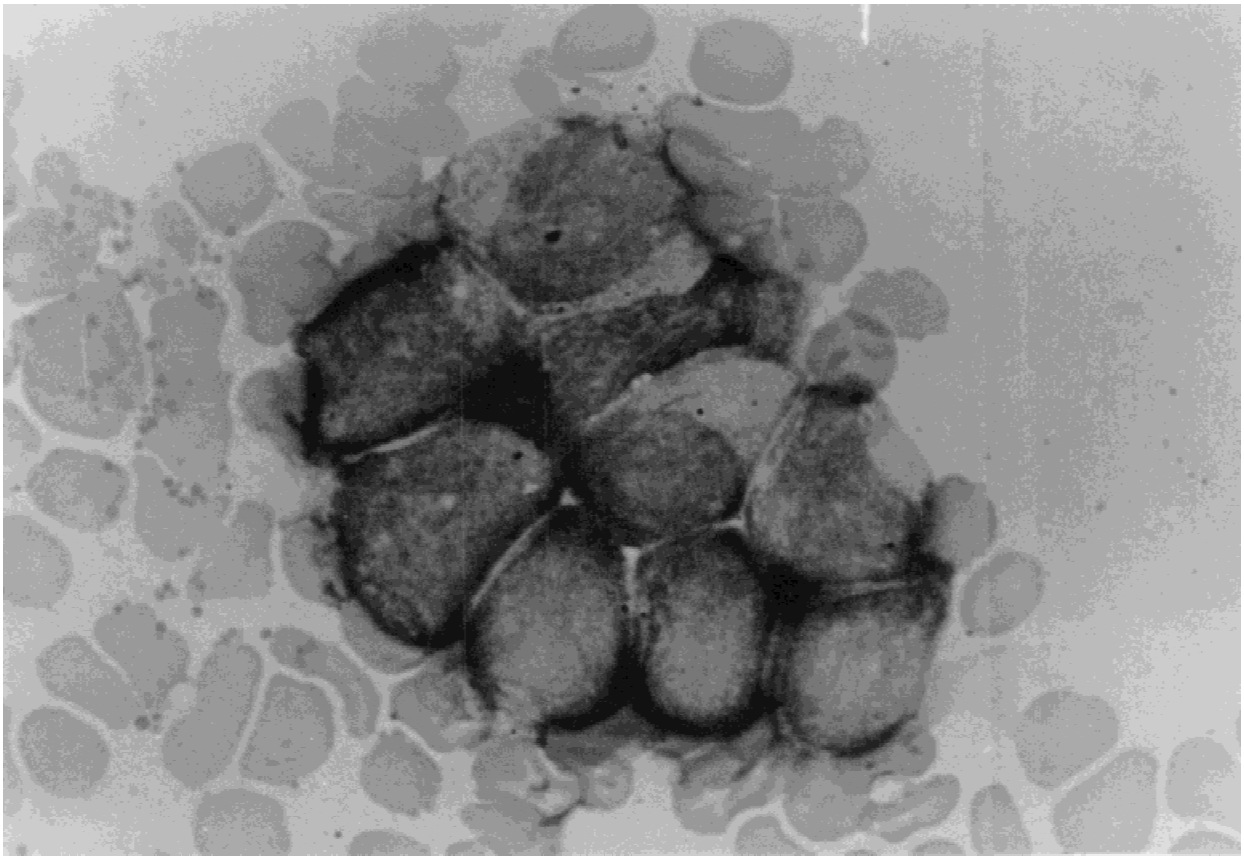

Fig. 1. BM: AML M2 (May-Grünwald-Giemsa stain, ×1,000).

Complete blood count (CBC) revealed: Hb 8.4 g/dl, platelet count 103.000/mm^3, white blood cell (WBC) count 19.000/mm^3 with 31% myeloblasts, 4% promyelocytes, 20% neutrophils, 40% lymphocytes, 5% monocytes.

BM aspirate showed more than 90% blasts resembling M2 myeloblasts without Auer rods (Fig. 1). Myeloperoxidase was positive. Immunological studies showed CD33 expression as myeloid marker.

A spinal tap was performed. Spinal fluid showed 230 cells/mm^3, protein 109 mg/dl, glucose 38 mg/ml. A cytocentrifuge preparation showed a uniform population of leukemic cells (Fig. 2). Myeloperoxidase was positive. Immunological studies were not performed.

Radiological examination and computed tomographic (CT) scan revealed in the right mandible a soft tissue mass without a destructive bone lesion. A biopsy of the mass was not performed. Chest X-rays and skeleton survey were normal.

On the basis of the above, intrathecal chemotherapy was begun every other day, with methotrexate, ara-C, and prednisone, resulting in a rapid disappearance of the leukemic cells. Then systemic chemotherapy was began according to the schedule 7 + 3 (ara-C + daunomycin) with vincristine and prednisone as induction therapy. During hematologic toxicity, the child experienced seizures; meningitis due to *Candida tropicalis* was diagnosed and she died a few weeks later.

Cytogenetic analysis was performed on BM cells and on spinal fluid cells after short-term culture (24 hr). Metaphases were analyzed after R(RBG) banding. All 50 metaphases from spinal fluid cells had normal female karyotype (46, XX). Trisomy 8 was detected in 49 of 50 metaphases from BM cells.

Cytogenetic studies of cultured skin fibroblasts were performed and a diploid number was found.

DISCUSSION

There have been scanty reports on karyotypic evolution in AML. In a series of 50 patients, 8 (16%) showed karyotypic evolution in serial cytogenetic BM samples [4]. Evolution of BM karyotype was, in a subsequent study, observed in 17 patients of 60 and, in particular, in 7 patients who were initially normal [5]. A +8 aberration was seen in 4 of 7 initially normal patients and 6 of 10 initially abnormal patients. More recently, 61% of 103 patients with AML underwent a change in karyotype [6]. Trisomy 8 aberration appears as the single most frequent

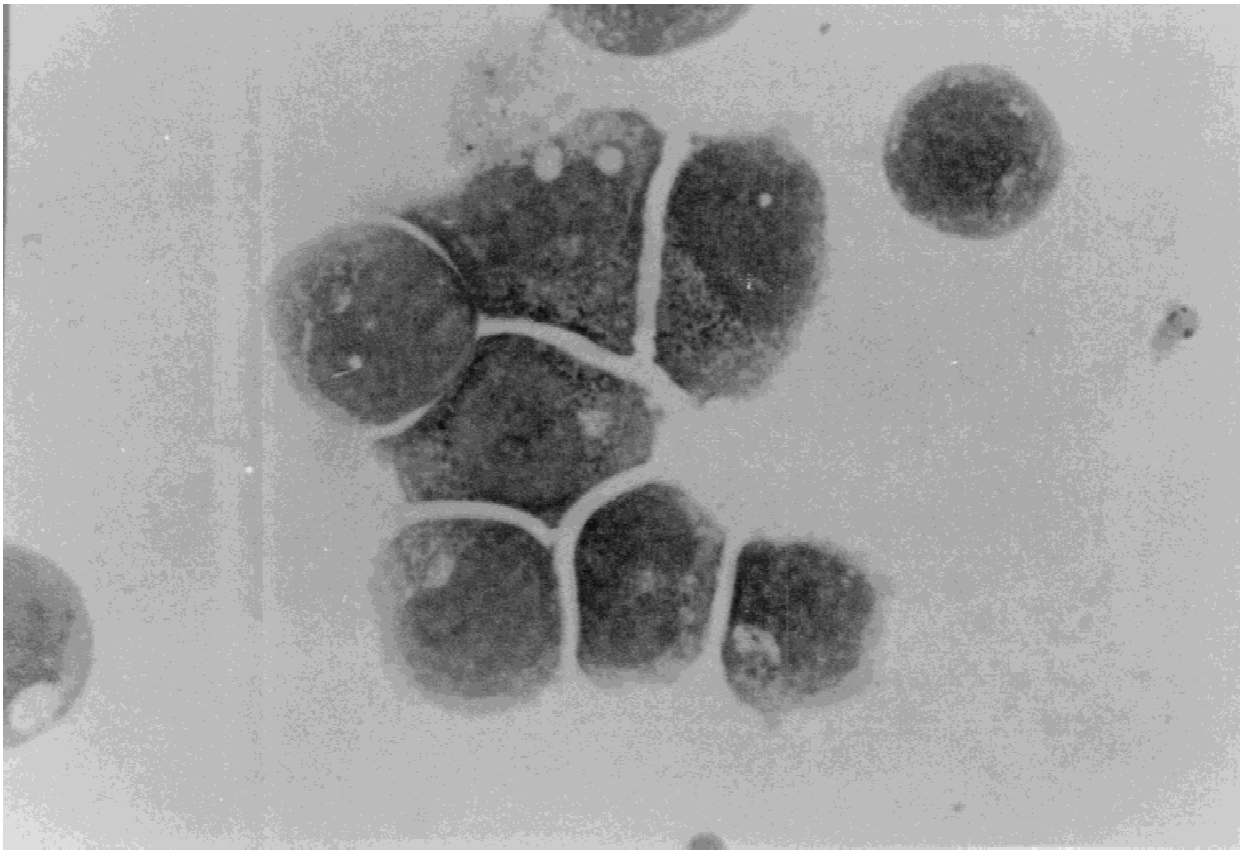

Fig. 2. Cell centrifugation of cerebrospinal fluid (May-Grünwald-Giemsa stain, ×1,000).

abnormality observed at diagnosis and in many patients the additional chromosome 8 represented the initial evolutionary change [5]. Stepwise gains of a single chromosome often occur during the clonal evolution of tumor cell populations sequentially investigated [7], and evolutionary changes appear to indicate the emergence of a resistant cell line. If the changes are present in the majority of the cells, they are often associated with a poor prognosis [5].

The present case shows the *simultaneous presence* at diagnosis of a 46 cytogenetic line in the CNS spinal fluid and a 47 (+8) cell line in the BM present *in the great majority* of the metaphases examined. Given the convincing evidence for a clonal origin of the leukemic cells from a single malignant mutant [8,9], it may be safely assumed that the blastic stem line was the same in the cerebrospinal fluid (CSF) and in the BM, as previously observed in ALL cases as well [3]. Furthermore, it is well established that, in a significant percentage of AML cases, microscopic leukemic foci are already present at the time of diagnosis. These observations support the view that the time sequence of chromosomal abnormalities reported in patients showing clonal evolution *after* diagnosis may have occurred in this case *before* diagnosis, with a leukemic population first appearing in the CNS and subsequently in the BM, where it is presumed that a single cell with trisomy 8 derived from the CNS compartment gave rise to a leukemic line that replaced the normal BM population. The absence of metaphases with trisomy 8 karyotype in the CNSL population might indicate that in the CNS microenvironment growth conditions for a more malignant leukemic subclone were not as good as in the BM.

As a more speculative hypothesis, the 46 malignant clones could have originated in the BM and have spread to the CNS. A more aggressive +8 subclone might have appeared in the BM, which could not have developed in the CNS, again because the microenvironment growth conditions were not favorable.

Whatever pathogenetic mechanism is involved, the main significance of the case described lies in the identification of a leukemic population in the CNS compartment cytogenetically distinct from the BM cell population, which shows a clonal evolution. The CNSL population, without apparent clonal evolution, may thus represent a less malignant process compared to the BM leukemic population.

The concept of a lower malignancy of the leukemic population in the CNS disease of AML easily fits in with the evidence of a less malignant course of this compli-

cation in AML. In fact, although the incidence of CNS involvement at diagnosis is definitely high in childhood AML patients, this complication at diagnosis did not appear to adversely affect the remission induction rate and the length of complete remission [2]. In cases of AML meningeal leukemia, an extremely rapid reduction of the leukemic cell number has been observed following intrathecal chemotherapy [2]. Furthermore, CNS ″prophylaxis″ with cranial radiation and intrathecal chemotherapy alone were successful in reducing CNSL incidence in childhood AML but survival was not apparently affected [2,10]. However, a lower risk for hematologic relapses and a higher cure rate were observed in childhood AML following ″preventive″ cranial radiotherapy, but this occurred only in low-risk AML children [11]. It is possible that in these cases, if a more malignant clone has not yet developed in the CNS, cranial radiotherapy would then eliminate the potential source of BM reseeding with the consequent systemic relapse.

Further parallel cytogenetic studies of spinal fluid and BM leukemic cells are needed in order to confirm the existence of a less malignant leukemic clone in the CNS of children with AML and to clarify the relationships between the leukemic populations of the two compartments, CNS and BM, in a possible multistep process of leukemogenesis. New techniques, such as florescent in situ hybridization, may facilitate the identification of chromosomal abnormalities not detectable by standard cytogenetic methods of investigation.

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